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Repellency effect of white flesh *Citrus grandis* osbeck fruit peel extracts against *Aedes aegypti* (Linn.) Mosquitoes

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Abstract
In many parts of the world, natural plant products have been traditionally used to repel mosquitoes. In this study, various *Citrus grandis*'s fruit peel extracts ((hexane, ethyl acetate, methanol and essential oil) have been tested to determine its repellency effect against ovipositing female and blood hungry female *Aedes aegypti* mosquitoes. The finding showed that oviposition activity by gravid female mosquitoes was successfully deterred by ethyl acetate extract at 10 ppm. In another study, pure essential oil effectively repelled female *Ae. aegypti* mosquitoes from attacking the bait in modified tunnel test. In addition, animal model applied with 20% essential oil showed good protection rate (94.7%). These results indicated that *Citrus grandis*'s fruit peel extracts have repellency effect on female *Ae. aegypti* mosquitoes.

Keywords: *Citrus grandis, Aedes aegypti*, oviposition, biting repellency

1. Introduction

Chemicals can protect human from vector mosquitoes using three different ways, namely irritation, repelling or killing [1]. A number of research work has focused on the synthetic chemicals [2, 3] designed to control mosquito population and disease transmission [4]. However, new approaches using natural products to control destructive insects and vectors of diseases is necessary due to the prevalence of vector resistance to synthetic insecticides, problem of toxicity, non-biodegradable effects and harmful effects on non-target organisms [5].

The sense of smell (olfaction) is one of the most important senses of mosquitoes [6] that they rely on for maintaining of their life. For example, they use their olfactory cues locating egg-laying (oviposition) sites, food source (nectar) and human hosts [7]. A number of compounds secreted by humans, such as carbon dioxide (human breath), lactic acid (from human skin and exhaled breath) and 1-octen-3-ol (human sweat and breath) have been shown as mosquito attractants [8].

Studies have shown that insect repellents use a similar mode of action. Each repellent binds and interacts with specific insect odorant and gustatory receptors changing their activity and thereby exerting their deterrent effects [9]. The most commonly used repellent is N, diethyl-m-toluamide (DEET) and has been in use for almost 50 years with 200 million users worldwide [10] due to its effectiveness as personnel protection [11]. However, a number of serious reactions (skin irritation and dermis infection) including fatal caused by the application of DEET has been reported [11, 12, 13]. Hence, botanicals are being explored to as an efficient line of defence against the vector mosquitoes and successful alternative to synthetic insecticides [14].

Insufficient water supply and inadequate waste management systems has also consequently led to a rise in mosquito breeding sites. These have led to an explosion in the mosquito-borne diseases infecting over 700, 000, 000 people globally [5]. Preventing egg laying or oviposition activity could disrupt life cycle of vector and subsequently inhibit population growth and pathogen dissemination. Therefore, potential mosquito breeding sites could be treated with oviposition deterrent agents as part of integrated vector management strategies [16, 17].

In many parts of the world, natural plant products have been traditionally used to repel mosquitoes [18].

Some botanical extracts act as toxicant on both adult as well as larval stages of mosquitoes, while others interfere with growth or with reproduction or produce an olfactory stimulus, hence acting as repellents and oviposition attractants [19, 20, 21, 22, 23, 24, 25]. Many plant extracts and oils repel mosquitoes, with their effect lasting from several minutes to several hours [26].
The repellency effect of *Citrus grandis* has been studied by several authors [27, 28]. However, most of the studies concentrated on essential oil and not emphasized on *Aedes aegypti*. Thus, this study was undertaken to investigate the potential of various *Citrus grandis* fruit peels extracts in preventing egg laying activity as well as repellence of biting activity using tunnel test and topical application on animal model against female *Aedes aegypti* mosquitoes.

2.0. Materials and methods

2.1. Plant collection and extraction

White flesh pomelo or *Citrus grandis* fruits were purchased from trusted vendor in Taiping, Perak. The fruits were brought to Herbal Processing Lab, Integrative Medicine Cluster, Advanced Medical and Dental Institute, USM for processing. A voucher of specimen (Referral number: MANO 2013-01) was deposited in the Herbarium Unit, Forest Research Institute Malaysia, Selangor, Malaysia. The fruit peels of white flesh pomelo has been processed, extracted (crude methanol extract, hexane fraction, ethyl acetate fraction, queous fraction and crude water and essential oil) as described in Manorenjitha *et al*. [29].

2.2. Preparation of stock solutions

One gram of crude methanol extract, ethyl acetate fraction, hexane fraction, aqueous fraction and crude water extracts was first dissolved in 100 ml of respective solvent (stock solution). Similarly, 1 ml of essential oil was dissolved in 100 ml of acetone (stock solution). From the stock solution, serial dilution was prepared.

2.3. Bioassay on oviposition repellency

This method was adapted from Phasomkusolsil and Soonwera [30] with a slight modification. For this study, 5 mosquito cages were prepared (each cage represents a replicate). Each cage (30 x 30 x 30 cm) contains 6 petri dishes (5: concentrations and 1: control) and a filter paper (folded into a cone shape). Five concentrations ranging from 0.1, 1, 10, 100, 1000 ppm were prepared by adding 0.5 ml of solution from each dilution into the petri dish filled with 4.5 ml of dechlorinated water. For control, 0.5 ml of acetone was added to 4.5 ml of dechlorinated water. Twenty gravid female *Ae. aegypti* mosquitoes (3 days after blood feeding) were randomly selected and transferred to each mosquito cage. The position of the petri dish with extracts was placed alternately at every replicate so as to nullify any effect of position on oviposition. After 48 hours, the number of eggs laid in the treated and control cups were counted under a microscope.
The oviposition activity index (OAI) was calculated using the following formula [31]:

$$OAI = (NT-NC)/ (NT+NC)$$

The OAI ranges from -1 to +1 with the positive index values indicating that the test solutions were attractants, the negative index values indicate that the test solutions were deterrent and 0 indicate neutral response.
The effective repellency (ER) of each test solution of an extract was calculated using the following formula [32]:

$$ER (\%) = (NC- NT) / NC \times 100\%$$

The effective attractancy (EA) of the test solution of an extract was calculated using the following formula [33]:

$$EA (\%) = (NT- NC) / NT \times 100\%$$

Where, NC is the number of eggs in the control cup; and NT is the number of eggs in the treatment cup.

2.4. The efficacies of nets impregnated with four *C. grandis* fruit peel extracts tested using modified tunnel test method

This method was adapted from WHO (2005) [34] with slight modifications. The test was conducted in modified chambers. A holding chamber measuring 40 cm (L) × 18.7 cm (W) × 18.7 cm (H) (a modified plastic container) is connected to a treatment chamber (mosquito cage; 30 cm (L) x 30 cm (W) x 30 cm (H)) via a tunnel (plastic tube; 30 cm (L) x 12 cm (D) with a trap door at one end. A removable cardboard frame attached with a piece of cloth (polyster net) measuring 15 cm x 15 cm with 9 holes measuring 1 cm (diameter) was fitted at one end of the tunnel (exit to treatment chamber). A cylindrical wire cage (measuring, diameter= 7 cm; length= 15 cm) covered with a mesh cloth was used to house a male rat (approximately 200 g in weight). The cylindrical cage was placed in the middle of the treatment chamber during the experiment. Treatment of 1 ml of acetone containing 6.25, 12.5, 25, 50, 75 and 100% (pure oil) of extract were applied on the net. As for the control, 1 ml of acetone was applied on the net. The nets drenched with solution were air dried 30 minutes before the experiments started. Fifty 5-7 days old blood starved female *Ae. aegypti* mosquitoes were released into the holding chamber at 1730. Readings were started as soon as the trap doors were released. Readings were recorded at every 30 minute intervals until 3 hours (last reading).
The percentage of repellency was calculated by the following formula.

$$\% \text{ Repellency} = [(T_a - T_b)/T_a] \times 100$$

Where, T_a is the number of mosquitoes in the holding chamber; and T_b is the number of mosquitoes in the treatment chamber.

2.5. Repellency effects of different concentration of *C. grandis* essential oil against female *Ae. aegypti* mosquitoes

This method was adapted from Oshaghi *et al.* [35] with a slight modification. Based on the results from previous study, the most effective extract that gave remarkable repellency in section 8.2.2 was selected for this study. Fifty 5-7 days old blood starved female *Ae. aegypti* mosquitoes were released into a mosquito cage measuring 30 cm x 30 cm x 30 cm. Rats (≈ 100 g) were anesthetized with pentobarbital (50 mg/kg b.w) before its abdomen was shaved. Treatment of 1 ml of acetone containing 5%, 10% and 20% of extract were applied on its exposed abdomen. DEET and acetone was used as the positive and negative control, respectively. At the start of the experiment, fifty 5-7 days old blood starved female *Ae. aegypti* mosquitoes were released into a mosquito cage (30 cm x 30 cm x 30 cm). The stopwatch was immediately started as soon as the rat was placed on top of the mosquito cage where the treated surface was exposed to the mosquitoes. The total number of bites was recorded for 2 minutes at each interval. The control and treatment tests were conducted simultaneously. Each test was replicated 3 times. Readings were recorded at every 30 mins until 3 hours (last reading).

The percentage of protection was calculated by the following formula.

% Protection = $[(T_a - T_b)/T_a] \times 100$

Where, T_a is the number of bites in the controlled rat; and T_b is the number of bites in the treated rat.

2.6. Statistical analysis

Data was expressed as mean ± S.E (Standard Error). The data obtained was analysed for normality test. When appeared not to be normally distributed, the data was subjected to a non-parametric analysis using SPSS version 20.

3.0. Results and Discussion

3.1. Bioassay on oviposition repellency

It has been proven that dengue virus distribution involves transovarial transmission [36]. Hence preventing egg laying and hatching is the one of the most important strategy in controlling of the disease [17]. In this study, two aspects of repellency were studied. The first is prevention of egg laying or oviposition deterrent and the second is prevention of biting. The results for oviposition are summarised in Table 1. Among six *Citrus grandis* fruit peel extracts tested only ethly acetate and hexane fraction showed reduction in the number of eggs deposited by gravid females *Ae. aegypti* mosquitoes. The mean number of eggs laid in 0.1 ppm and 1 ppm concentrations of ethyl acetate were 45.4 and 33.6 eggs per cup respectively, while at the higher concentrations (10 ppm, 100 ppm and 1000 ppm) recorded no eggs were laid in all the test cups. In the hexane treated cups, the mean number of eggs laid for all 5 concentrations (0.1 ppm, 1 ppm, 10 ppm, and 100 ppm) were 427.2, 350.2, 191.4 and 21.4 eggs per cup respectively, while no eggs were laid in the test cup treated with 1000 ppm hexane. Essential oil extract showed reduction in the number of eggs for concentrations 10 ppm, 100 ppm and 1000 ppm, while for lower concentrations (0.1 ppm and 1 ppm) high number of eggs were recorded. As for methanol, water and aqueous extracts, higher and lower concentration did not affect the number of eggs deposited.

The current study showed evidence of oviposition deterrent by white flesh *Citrus grandis* fruit peel extracts. In general, methanol crude extract, ethyl acetate fraction, hexane fraction (except 0.1 ppm), aqueous fraction, water crude extract and essential oil (except 0.1 and 1 ppm) displayed oviposition deterrent activity. However, among the six extracts, ethyl acetate fraction exhibited remarkable oviposition deterrent at lower concentration (10 ppm). The findings of this study are in conformity with the results reported by several researchers. Decrease in egg laying between 18% to 99% was detected for female *Anopheles stephensi* after exposure to ethanolic leaf extract of *Solanum trilobatum* (purple fruited pea eggplant) at concentration ranging from 0.01, 0.025, 0.05, 0.075 and 0.1% [37]. Similarly, *An. stephensi* treated with ethanolic extract of *Andrographis paniculata* (king of bitters) recorded oviposition active index (OAI) values of -0.28, -0.45, -0.49 and -0.59 for extract concentration of 29, 35, 41 and 46 ppm, respectively [38]. At 100 ppm (the highest test concentration), the acetonilic extracts of leaves of *Eugenia jambolana* (Indian blackberry), *Solidago canadensis* (meadow goldenrod), *Euodia ridleyi* (evodia) and flower of *Spilanthes mauritiana* (spilanthes) were reported to show maximum oviposition deterrent effect against female *Ae. aegypti* (-0.71, -0.71, -0.90,-0.93), *An. stephensi* (-0.85, -0.91, -1.00, -1.00) and *Culex quinquefasciatus* (-0.81, -0.84, -1.00, -1.00) [39]. Likewise Ahbirami *et al*. [17] noted that acetone extract of *Ipomoea cairica* (railroad creeper) leaves have successfully deter the oviposition activity at test concentrations of 50, 100, 450 ppm

Table 1: The oviposition deterrent/attractant/ neutral of 6 extracts tested against *Ae. aegypti* after 48 hours of exposure

Extract	Conc. (ppm)	Number of eggs ± SD	OAI	ER%	EA%
Hexane	Control	418.4 ± 44.76a			
	0.1	427.2 ± 32.04a	0	-	2.1
	1	350.2 ± 83.06a	-0.1	16.3	-
	10	191.4 ± 67.85b	-0.4	54.3	-
	100	21.4 ± 13.39c	-0.9	94.9	-
	1000	0d	-1.0	100	-
Ethyl Acetate	Control	317 ± 95.18a			
	0.1	45.4 ± 59.53b	-0.7	85.7	-
	1	33.6 ± 47.65b,c	-0.8	89.4	-
	10	0c	-1.0	100	-
	100	0c	-1.0	100	-
	1000	0c	-1.0	100	-
Methanol	Control	426.60 ± 60.2a			
	0.1	373 ± 56.42a,b	-0.1	12.6	-
	1	331.60 ± 42.28b	-0.1	22.3	-
	10	296.4 ± 56.33b,c	-0.2	30.5	-
	100	271.4 ± 32.24c	-0.2	36.4	-
	1000	208.8 ± 31.95d	-0.3	51.1	-
Essential Oil	Control	365.8 ± 57.77a			
	0.1	452.20 ± 37.12b	0.1	-	23.6
	1	460 ± 21.71b	0.1	-	25.8
	10	244.4 ± 76.70c	-0.2	33.2	-

	100	76.8 ± 17.8d	-0.7	79.0	-
	1000	22.8 ± 9.86e	-0.9	93.8	-
Water	Control	467.8 ± 64.77a			
	0.1	428.4 ± 80.13a	-0.04	8.4	-
	1	267 ± 42.81b,c	-0.3		42.9 -
	10	354.2 ± 102.22a,c	-0.1	24.3	-
	100	292.2 ± 82.77c	-0.2	37.5	-
	1000	272.4 ± 79.55c	-0.3	41.8	-
Aqueous	Control	482.2 ± 51.45a			
	0.1	411.2 ±114.93a,b	-0.1	14.7	-
	1	287.2 ± 50.68b	-0.3	40.4	-
	10	222 ± 50.35c,d	-0.4	54.0	-
	100	274.8 ± 27.78b,c	-0.3	43.0	-
	1000	191.8 ± 41.70d	-0.4	60.2	-

Mean number of eggs laid followed by the same letters within the same columns are not significantly different (P>0.05, non parametric Mann-Whitney U Test). n=5.

The OAI ranges from -1 to +1; the positive index values (+) indicated that the test solutions were attractants; the negative index values (-) indicated that the test solutions were deterrent and 0 indicating neutral response. OAI= Oviposition Active Index; ER= Effective Repellency; EA= Effective Attractancy

when tested against *Ae. aegypti* (-0.97, -1.00, -1.00) and *Aedes albopictus* (-0.69, -0.98, -1.00). In another study, Yadav *et al*. [40] investigated the effect of acetone extracts of *Callistemon viminalis* (weeping bottlebrush), *Hyptis suaveolens* (pignut), *Prosopis juliflora* (mesquite), *Vernonia cinerea* (French bouton violet) on oviposition activity index against female *Ae. albopictus* at concentration ranging from 50, 100 and 200 ppm. They found that *Prosopis juliflora* demonstrated strong oviposition deterrent effect at 100 ppm (-0.466). In another study, Warikoo and Kumar [14] evaluate the effect of *Argemone mexicana* (prickly poppy) leaf extracts (petroleum ether, hexane, benzene, acetone and ethanol) at concentrations ranging from 40, 60, 80, 100, 200, 400, 600, 800 and 1000 ppm tested against gravid female *Ae. aegypti* mosquitoes. They found that petroleum-ether at 400 ppm showed the highest deterrent of oviposition activity (-0.93).

Oviposition is one of the most important aspects in the life cycle of mosquitoes, and preventing oviposition will reduce their population [41]. The prevention of oviposition activity in female *Ae. aegypti* mosquitoes by various extracts of *C. grandis* fruit peel showed that the female mosquitoes are sensitive to chemical stimuli and respond to the odour of the extracts [42]. The oviposition activity index (OAI) of six extracts at 5 concentrations when being compared to the control ranged from 0 to -1.0 for hexane extract, -0.7 to -1.0 for ethyl acetate, -0.1 to -0.3 for methanol extract, 0.1 to -0.9 for essential oil, -0.04 to -0.3 for water extract and -0.1 to -0.4 for aqueous extract, respectively. Based on the results, testing cups treated with 10 ppm of ethyl-acetate extract and 1000 ppm of hexane extract of *C. grandis* fruit peels showed maximum oviposition deterrent.

Table 2: The efficacies of nets impregnated with four *C. grandi*s fruit peel extracts at 3 hours after application

	ER (%) ± SD						
Extract \ Conc. (%)	**Control**	**6.25**	**12.5**	**25**	**50**	**75**	**100**
Hexane	4.0 ± 2.0a	11.8 ± 4.7b	13.1 ± 10.4a,b,c,d	19.5 ± 2.4c	19.5 ± 5.7b,c,d	13.8 ± 5.0b,c	28.5 ± 6.0d
Ethyl acetate	4.67 ± 1.2a	13.3 ± 5.2b	16.0 ± 7.2b,c	26.5 ± 18.8b,c,d	28.0 ± 5.6c,d	28.7 ± 8.3c,d	29.4 ± 4.0d
Methanol	2.67 ± 1.2a	8.86 ± 7.1a,b,c	10.3 ± 4.2b	19.2 ± 5.1b,c	20.5 ± 7.0c	22.7 ± 9.3c	27.3 ± 11.1b,c
Essential oil	3.33 ± 1.2a	7.61 ± 4.4b	43.4 ± 5.4c	62.7 ± 4.0d	68.2 ± 8.6d	88.3 ± 4.4e	100 ± 0f

Mean percentage of repellency followed by the same letters within the same rows are not significantly different (P>0.05, non parametric Mann-Whitney U Test). n=3, ER-Effective repellency

3.2 The efficacies of nets impregnated with four *C. grandis* fruit peel extracts

The efficacy of nets of impregnated with 4 extracts of *Citrus grandis* fruit peels were tested against blood hungry female *Ae. aegypti* and the results are summarised in Table 2. The results showed that essential oil was significantly more effective than extracts. Hexane, ethyl acetate and methanol extracts were not successful in deterring the hungry females from entering the chamber with the bait as it gave protection of less than 20% at higher concentrations. However, *Citrus grandis* essential oil showed the higher repellency effect with increase in concentrations. At the highest concentration (100%), pure essential oil gives 100% protection from mosquito bites.

It is noteworthy that the essential oil of white flesh *C. grandis* fruit peel effectively deterred mosquitoes from entering the chamber that contained the bait (rat). As the concentration of *C. grandis* essential oil increased, reduction in the number of mosquitoes entering the chamber occupied by the bait was observed. Total repellency was achieved by 100% (undiluted) essential oil which lasts up to 3 hours. This was followed by 75% essential oil which provided 88.3% repellency against hungry females *Ae. aegypti*. Mosquitoes were prevented from entering the chamber probably due to high vapour toxicity of the volatile phytochemicals presence in the oil [43, 44]. This finding is in agreement with several studies that found essential oil as effective repellents. For example, Kweka *et al*. [45] studied the repellency effect of citronella oil (as control), essential oil of *Ocimum suave* (wild basil) and *Ocimum kilimanscharium* (African blue basil) leaves (the netting material impregnated with 75 mg/m^2, 100 mg/m^2, 200 mg/m^2, 250 mg/m^2 and 500 mg/m^2 of each extracts) using tunnel test method with rabbit as bait. They found that the best repellency effect was demonstrated *by Ocimum suave* when tested against *Anopheles gambiae* (50%), *Anopheles arabiensis* (58%) and *Cx. quinquefascitus* (67%) at highest concentration (500 mg/m^2). Similar finding was also shared by Mukandiwa *et al*. [46] using leaf extracts (crude acetone extract, hexane fraction) and seselin (isolated compound) of *Clausena anisata* (horsewood) against the dengue vector, *Ae. aegypti*. At 15% concentration crude acetone extract and hexane fraction showed moderate protection of 46.89 and 50.13%, respectively after 3 hours of exposure. In contrast, seselin did not repel female *Ae. aegypti* mosquitoes from entering the chamber containing bait.

Table 3: Repellency effect of different concentration of *C. grandis* essential oil against female *Ae. aegypti* mosquitoes at 3 hours after application.

Concentration (%)	Percentage of Repellency (mean ± SD)	Ratio to DEET
Control	0 d	0
DEET	97.9 ± 2.1a	1
5% EO	52 ± 5.8c	0.53
10% EO	62.5 ± 6.2c	0.64
20% EO	94.7 ± 0.9b	0.97

Mean percentage of repellency followed by the same letters within the same rows are not significantly different (P>0.05, non parametric Mann-Whitney U Test). n=3, EO- essential oil

3.3. Repellency effect of different concentration of *C. grandis* essential oil against female *Ae. aegypti* mosquitoes

The results of the laboratory study on rats comparing control (acetone), DEET and 3 different concentrations of *C. grandis*'s fruit peel essential oil are presented in Table 3. The results showed that with the exception of the control, the 10% Malaysian Standard DEET (positive control) and the 3 essential oil concentrations exhibited repellency activities. DEET was found to be the most effective with 97.9% repellency followed by 20% essential oil which recorded 94.7% protection against female *Ae. aegypti* from biting the rats. At the same time, 5% and 10% essential oils demonstrated repellency effect with moderate protection of 52% and 62.5%, respectively.

In the present study, 20% essential oil provided 94.7% protection of the test animal against female *Ae. aegypti* bites up to 3 hours. The percentage of repellency is slightly lower than the 15% DEET. It is possible that the responsible compound or compounds are stable and did not vaporise substantially for the extracts to lose their activity over the 3 hours test period [46]. The present study is in agreement with several researchers who have documented the efficacy of essential oil from plant as the best protection against insects bites. Kiplang'at and Mwangi [47] investigated the repellency effect of *Azadirachta indica* (neem) oil, *Ocimum basilicum* (sweet basil) oil, *Eucalpytus citriodora* (lemon eucalyptus) oil and *Chrysanthemum cinerariaefolium* (chrysanths) extract were evaluated using rabbit as bait against female *Ae. aegypti* mosquitoes bites. They reported that *Chrysanthemum cinerariaefolium* extract at concentration 0.002%, 0.005% and 10% *Azadirachta indica* recorded mean repellency of 81.58%, 85.94% and 85.79% respectively. On top of that, combination of essential oils consisted of *Azadirachta indica* oil, *Ocimum basilicum* oil and *Eucalpytus citriodora* oil with ratio 1:1:1 were found to provide complete protection against female *Ae. aegypti* mosquitoes bites. Similarly, Kiplang'at [48] demonstrated that formulation consisted of *Ocimum basilicum* oil, *Eucalpytus citriodora* oil, *Azadirachta indica* oil (each 10% with ratio 1:1:1) and 1 mg/ml of crude oleoresin extract from *Chrysanthemum cinerariaefolium* exhibited total protection of the test animal (rabbit) when tested against female *Ae. aegypti* bites. In another study, Mukandiwa *et al*. [46] compared the repellency efficacies of *C. anisata* extract (acetone) and its sub-fractions (butanol, chloroform and hexane) with 15% DEET using guinea pig as bait and female *Ae. aegypti* as the test insects. Their study revealed that acetone crude extract at 15% (concentration) and hexane fraction at 7.5% (concentration) recorded an average repellency of 93% and 67%, respectively over the 3 hours of test period. Likewise, essential oils of *Cymbopogon citratus* (lemongrass) and *Tagetes minuta* (Mexican marigold) with concentration ranging from 0.125, 0.250, 0.500, 0.750 and 1 mg/ml was tested using hamster as bait against *Phlebotomus duboscqi* (sandfly) bites. Strong repellency effect was observed for both *Cymbopogon citratus* and *Tagetes minuta* essential oil at the highest concentration (1 mg/ml). In addition, *Cymbopogon citratus* was found to provide complete protection to the hamster which last up to 3 hours [49].

4.0. Conclusion

The findings indicated that white flesh *C. grandis* fruit peel extracts contained dual properties as an oviposition deterrent and biting repellent. The presence of chemical constituents in certain extracts could play a crucial role in deterring the oviposition activities. This study recommends the oviposition deterrent activity of ethyl acetate fraction of *C. grandis* fruit peel to be further studied under field condition. At the same time, the positive results obtained from tunnel test and repellent test using animal as bait is indicative for further study using human volunteers to determine *C. grandis* fruit peel essential oil potency as an alternative to DEET.

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5.0. References

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